

## ORIGINAL ARTICLE

# Vitamin B12 in metformin-treated diabetic patients: a cross-sectional study in Brazil

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## SUMMARY

**Objective:** The objective of this study was to evaluate the presence of vitamin B12 deficiency and the factors associated with serum vitamin B12 levels in a sample of metformin-treated Brazilian diabetic patients. **Method:** Cross-sectional study. **Results:** 144 patients were included. Serum vitamin B12 levels were low (< 125 pmol/L) in 10 patients (6.9%) and possibly low (125 - 250 pmol/L) in 53 patients (36.8%). Serum vitamin B12 levels were negatively associated with age ( $B = -3.17$ ;  $\beta = -0.171$ ;  $p = 0.037$ ) and duration of metformin use ( $B = -33.36$ ;  $\beta = -0.161$ ;  $p = 0.048$ ), and positively associated with the estimated intake of vitamin B12 ( $B = 67.96$ ;  $\beta = 0.249$ ;  $p = 0.002$ ). **Conclusion:** The present findings suggest a high prevalence of vitamin B12 deficiency in metformin-treated diabetic patients. Older patients, patients in long term treatment with metformin and low vitamin B12 intake are probably more prone to this deficiency.

**Keywords:** *Diabetes mellitus*; vitamin B 12; metformin.

Study conducted at Serviço de Medicina Interna, Hospital de Clínicas de Porto Alegre, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS

**Submitted on:** 10/11/2010

**Approved on:** 10/27/2010

### Financial Support:

Fundo de incentivo à Pesquisa e Eventos do Hospital de Clínicas de Porto Alegre, Porto Alegre, RS, Brasil

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**Conflict of interest:** None.

## INTRODUCTION

Vitamin B12 deficiency can lead to hematological abnormalities, cognitive disorders, and neuropathy<sup>1</sup>. This vitamin is obtained from animal origin food, so its deficiency may be related to eating habits, changes in the absorption or metabolic mechanisms.

Metformin is the first choice of treatment for type 2 diabetes mellitus (DM2), decreasing insulin resistance, and cardiovascular and mortality risks. It is usually well tolerated, although it may cause gastrointestinal side-effects<sup>2</sup>.

The association of metformin with vitamin B12 deficiency has been studied for some time, yet, the mechanism leading to this deficiency is not fully understood<sup>3,4</sup>. Recently, vitamin B12 deficiency has been associated with neuropathy in metformin-treated diabetic patients<sup>5</sup>.

A literature review was made in the PubMed and LILACS databases combining the keywords: *vitamin B12 and metformin* and no studies with Brazilian patients were identified until August, 2010. Given the importance of vitamin B12 deficiency, this study aimed to estimate its prevalence and to evaluate factors associated with serum vitamin B12 levels in metformin-treated DM2 patients, in the outpatient setting of a tertiary hospital in Southern Brazil.

## METHODS

All DM2 patients treated with metformin for more than one year in the ambulatory of Internal Medicine at the Hospital de Clinicas of Porto Alegre were invited to participate, until completion of the sample. Patients with partial or total gastrectomy, Crohn's disease, disorders of the terminal ileum and using supplemental vitamin B12 were excluded. Factors evaluated in the study were age, gender, weight, metformin dosage, duration of metformin use, dietary intake of vitamin B12, red cell mean corpuscular volume (MCV), glycosylated hemoglobin and use of other medications or supplements.

Sample size was set to 147 patients estimating a 25% prevalence of vitamin B12 deficiency, with an accuracy of 7%, and  $p < 0.05$ .

Intake of vitamin B12 was estimated by the 24-hour food recall method. The book *Álbum fotográfico de porções alimentares*. (Photographic Record for Dietary Inquiries): *appliances and parts* was used to illustrate the food portions<sup>6</sup>. A table was used to convert approximate cooking measurements into grams<sup>7</sup>. Based on this information, intake of vitamin B12 was estimated using a chart containing the amount of vitamin B12 in food in Brazil<sup>8</sup>. Vitamin B12 intake was considered adequate or not according to the *Dietary Reference Intake* (DRI) for vitamin B12, as proposed by the Institute of Medicine of the USA<sup>9</sup>.

This study was approved by the Ethics Committee on Research of the Hospital de Clinicas de Porto Alegre and all patients signed an informed consent before entering the study. One blood sample from each patient was ob-

tained and serum samples were stored at  $-70^{\circ}\text{C}$ . Vitamin B12 levels were determined in a single batch by electrochemiluminescence - Modular E170 Roche. Vitamin B12 deficiency was defined by a serum level  $< 125$  pmol/L and was considered possible when the serum level of vitamin B12 ranged from 125 pmol/L to 250 pmol/L<sup>10</sup>.

Data analysis was performed with the Statistical Package for the Social Sciences (SPSS) version 16.0. Association of serum vitamin B12 levels with the factors studied was evaluated by Spearman's correlation test, Student's t test or the Mann-Whitney test. All factors associated with the serum vitamin B12 levels with  $p \leq 0.2$  were included in a linear regression model to determine the factors independently associated, as suggested by Altman<sup>11</sup>. The variables without normal distribution were transformed into their natural logarithms. Values of  $p < 0.05$  were considered statistically significant.

## RESULTS

In the period from July 3 to August 13, 2009, 158 eligible patients were identified and all agreed to participate. Two patients were excluded for reporting irregular use of metformin. Twelve patients were excluded for not undergoing blood collection. The analysis was done based on data obtained from 144 patients. Characteristics of these patients are shown in Table 1.

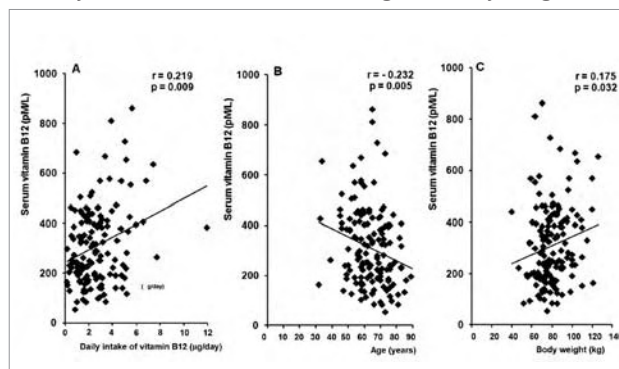
Serum vitamin B12 levels were low ( $< 125$  pmol/L) in 10 patients (6.9%) and possibly low (125 pmol/L to 250 pmol/L) in 53 patients (36.8%) and were associated with age, estimated vitamin B12 intake and body weight, as shown in Figure 1. The association of metformin dose and duration of metformin use with serum vitamin B12 levels was, respectively,  $r = -0.114$ ,  $p = 0.173$  and  $r = -0.128$ ,  $p = 0.129$ . Sixty eight patients (47%) had adequate daily intake of vitamin B12 ( $\mu 2.4$   $\mu\text{g}$ ).

**Table 1** – Characteristics of metformin-treated diabetic patients (n = 144)

Age (years)	63.7 $\pm$ 11.30
Women n (%)	91 (63.2)
Weight (kg)	80.3 $\pm$ 15.4
Height (m)	1.62 $\pm$ 0.09
BMI (kg/m <sup>2</sup> )	30.62 $\pm$ 5.18
Time using metformin (years)	4 (2/8.1)
Dose of metformin (mg)	2550 (1700/2550)
Use of omeprazole n(%)	57 (39.6)
Use of calcium carbonate n(%)	17 (11.8)
Hb A1c (%)	7.70 (6.88/9.73)
Red cell MCV (fL)	88.0 $\pm$ 4.6
Daily intake of B12 ( $\mu\text{g}$ )	2.25 (1.36/3.38)
Serum vitamin B12 (pmol/L)	311.0 $\pm$ 155.5

Data are expressed as mean  $\pm$  standard deviation, median (25/75 percentile) or n (%).

**Figure 1** – Correlation of serum vitamin B12 levels in metformin-treated type 2 diabetic patients (n = 144) with A: daily intake of vitamin B12; B: age; C: body weight.



There was no association of serum B12 levels with gender [men (n = 54):  $311.6 \pm 146.3$  pmol/L and women (n = 90):  $310.6 \pm 161.6$  pmol/L,  $p = 0.97$ ], use of omeprazole [yes (n = 58):  $301.0 \pm 148.4$  pmol/L and no (n = 86):  $317.8 \pm 160.6$  pmol/L,  $p = 0.52$ ], use of calcium [yes (n = 17):  $298.7 \pm 204.4$  pmol/L and no (n = 127):  $312.6 \pm 148.8$  pmol/L,  $p = 0.79$ ] and red cell MCV ( $r = 0.035$ ,  $p = 0.68$ ) and glycosylated hemoglobin ( $r = 0.092$ ,  $p = 0.28$ ).

The adjusted model included age, estimated daily intake of vitamin B12, body weight, dose of metformin and duration of metformin use, excluding in two stages the less significant factors. Serum vitamin B12 levels were independently and negatively associated with age ( $B = -3.17$ ;  $\beta = -0.171$ ;  $p = 0.037$ ) and duration of metformin use ( $B = -33.36$ ;  $\beta = -0.161$ ;  $p = 0.048$ ), and positively associated with the estimated intake of vitamin B12 ( $B = 67.96$ ;  $\beta = 0.249$ ;  $p = 0.002$ ), as shown in Table 2.

## DISCUSSION

In the present study serum vitamin B12 levels were low and possibly low in, respectively, 6.9% and 36.8% of the 144 diabetic patients treated with metformin for more than one year. The prevalence of vitamin B12 deficiency varies according to the cutoff for serum vitamin B12 level used<sup>10,12</sup>. Ideally, the serum methylmalonic acid would be measured in patients with possibly low serum vitamin B12 levels<sup>1,13</sup>.

Our results are quite similar to other recent cross sectional studies, that included 195 metformin-treated DM2 patients<sup>4</sup>: 79 (40.5%) had serum vitamin B12 levels between 73.8 and 258.3 pmol/L. Forty-three of these patients had elevation of either methylmalonic acid or homocysteine levels.

Although most studies associate vitamin B12 deficiency to prolonged use of metformin, a randomized study involving 400 patients with DM2 showed that within only 16 weeks of metformin use, patients had their mean serum folate and vitamin B12 levels reduced in 7% and 14%, respectively, and a 4% increase in mean serum homocysteine levels<sup>14</sup>.

Since the only source of this vitamin is food, diet has great importance in the prevention of its deficiency, nevertheless, the lack of a strong correlation between estimated daily intake of vitamin B12 and serum vitamin B12 levels is possibly due to this micronutrient store in the liver<sup>13</sup>. The method used in our study to estimate vitamin B12 intake is also subject to error; furthermore some day to day variability in food intake is expected. Although estimating vitamin B12 content in food is not precise, it allowed us to assess an additional factor that could influence serum levels of this vitamin. Even though the South of Brazil is a region where red meat is an everyday food, most of the population evaluated had an estimated vitamin B12 intake below the minimum RDI. Nutritional deficiency could have contributed to the low serum vitamin B12 levels in our patients.

Serum vitamin B12 levels were inversely associated with age. Studies evaluating the elderly population of industrialized countries have shown prevalence of vitamin B12 deficiency of around 20%<sup>13</sup>, implicating age as a possible risk factor for vitamin B12 deficiency.

In this study, serum vitamin B12 levels were not associated with the dose of metformin, but were associated with treatment duration. In a case-control study that included 155 diabetic patients with vitamin B12 deficiency, both emerged as independent risk factors for vitamin B12 deficiency, after adjusting for potential confounding factors<sup>3</sup>. This discordance could be at least in part explained by the self-report method used in this study to determine the dose and duration of metformin use. Metformin has adverse effects such as diarrhea, nausea and change in taste<sup>15</sup>, which could cause underreported irregular use.

This study has important limitations. Its design only makes it possible to evaluate associations. The absence of a control group makes it impossible to compare prevalence of vitamin B12 deficiency in a similar population not using metformin, which may have vitamin B12 deficiency associated with other factors, such as described in the Framingham study<sup>16</sup>. Nevertheless, a recent randomized controlled clinical trial demonstrated an increased risk of vitamin B-12 deficiency in long term treatment with metformin<sup>17</sup>.

**Table 2** – Factors associated with serum vitamin B12 levels in metformin-treated type 2 diabetic patients (n = 144)

Variables	Multivariate analysis*			
	B	SE	$\beta$	p
Estimated vitamin B12 intake	67.957	21.894	0.249	0.002
Age	-3.172	1.505	-0.171	0.037
Metformin use duration	-33.358	16.733	-0.161	0.048

\*Model including vitamin B12 intake, age and metformin use duration, dependent variable: serum vitamin B12; adjusted  $R^2 = 0.111$

Although serum vitamin B12 level is the most used test to diagnose deficiency of this vitamin<sup>18,19</sup> due to its low cost<sup>19</sup>, its sensitivity is not very high. It can be in the normal range in a significant proportion of patients with disabilities in which serum homocysteine or methylmalonic acid levels allow the early identification of vitamin B12 deficiency<sup>18</sup>. The large number of metformin-treated DM2 patients at risk for vitamin B12 deficiency makes it important to have better tests to clearly diagnose this condition, prior to committing these patients to lifelong treatment with vitamin B12. Although our study did not answer several questions, we believe it is important to call the medical community's attention to this common problem.

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